STUDIES ON EXPLANTS OF PEPPER (*CAPSICUM* SP) TREATED WITH DESIGNATED HORMONES.

E.H. Kwon-Ndung* and Wachukwu, C.P

Department of Plant Science and Biotechnology,
Nasawara State University Keffi.
Current address: Department of Botany, Federal University Lafia.

Abstract

A study was conducted in the Biotechnology Advanced Laboratory (BAL) of Sheda Science and Technology Complex (SHESTCO) Abuja on the growth and yield of 4 pepper varieties (Capsicum annum L.) cultured on Murashige and Skog (MS) basal medium supplemented with different plant growth regulators (PGRs) at various concentrations. The PGRs included 0.3ml, 1.3ml, 2.5ml and 3.8ml of indole-3-butyric acid (IBA), 1.5ml, 2ml, 2.5ml and 3ml of Nicotinic acid and 0.5ml, 1ml, 1.2ml and 2ml of silver nitrate ($AgNO_3$). Regenerated plants were obtained from cotyledon explants of pepper varieties by a culture procedure. Parameters assessed include rate of germination, shoot elongation, rooting capacity and fruit yield. $AgNo_3$ significantly increased germination rate in different pepper varieties. Gibberellic acid (GA_3) was the key factor in shoot elongation. MS basal medium with indole-3-butyric acid (IBA) were easier to root for the highest numbers of root were obtained in MS with Nicotinic acid and fruit yield. An efficient protocol for regeneration of pepper through economically viable explants of indigenous pepper varieties was established.

INTRODUCTION

Pepper (*Capsicum sp)* an economically important vegetable and spice crop worldwide. It belongs to the genus, Capsicum, member of the nightshade family, Solanaceae. The genus Capsicum consists of approximately 22 wild species and 5 domesticated species (Bosland, 1994), domesticated for vegetable and industrial (Oleoresin and Capsaicin) purposes. Pepper is widely cultivated throughout the world, mainly in Asia, South and North America and part of Africa (Morrison *et al.*,1986).

The propagation of pepper through seeds is restricted by short span of viability and low germination rate. Since the plants also lack natural vegetative propagation, tissue culture methods provide a novel way for the asexual multiplication of pepper plant (Morrison *et al.*, 1986). Tissue culture technique in pepper lag behind most other vegetable crops, mainly due to its recalcitrance to regeneration (Diaz *et al.*, 2000). Low differentiation frequency, difficulty in shoot elongation, and low response (Dabauza and Pena, 2001), are main barriers to micro-propagation of pepper.

The objectives of this study are to develop a simple and efficient procedure for regeneration of pepper and determine the efficiency of selected PGRs in the regeneration process. In addition, it was also intended to create variability in local varieties of pepper through *in vitro* culture.

MATERIALS AND METHODS

The study was conducted in the Biotechnology Advanced Laboratory (BAL) of Sheda Science and Technology Complex (SHESTCO), Abuja between June and August in 2009. Local seeds of pepper varieties: Atarugu, Shumbo, Borkonu Kanana and Tatasai (Plate 1) were obtained from Keffi Market at Keffi Local Government Area of Nasarawa State and grown in SHESTCO in the greenhouse in plastic trays arranged in a randomized complete block design .

Plate 1: Local seed of pepper : Atarugu (top left), Shumbo(top right), Borkonu Kanana(bottom left) and Tatasai(bottom right) from Keffi market.

1mg/ml of $AgNO_3$ and 0.1mg/ml each of IBA, GA_3 and Nicotinic acid were prepared for the study. Murashige and Skoog (MS) basal media were prepared in four places using 1L (1000ml) beaker each, about 500ml of distilled water was poured into each of the beaker and placed on a magnetic stirrer with the magnetic bar inside the water. Four different media were prepared for this study. 1L each of Murashigea and Skoog basal medium were prepared into four places, 50ml/L of Macro salt + 5ml/L of micro salt + 30g/L of sucrose and 2.8g/L of Agar (geltrite) were dissolved into each of the beaker one after the other. pH of 5.8 were taken, using 1M hydrochloric acid (HCL) or 1M sodium hydroxide (NaOH). The media was made up to 1L (1000ml) in a graduated measuring cylinder using distilled water, each of 1L media prepared were divided into 4 (250ms each), different concentrations of the plant growth hormones were added into each of the 250mls media as follows: 0.3ml, 1.3ml, 2.5ml and 3.8ml of IBA were added into each of 250ml of the MS medium.

1.5ml, 2ml, 2.5ml and 3ml of Nicotinic acid were added into each of the 250ml of 1L MS medium. 0.5ml, 1ml, 1.2ml and 2ml of $AgNO_3$ were added into each of 250ml of the 1L MS medium and 2.5ml, 3.8ml, 5ml and 6.3ml of GA_3 were added into each of 250ml of the 1L MS medium. Each 250mls medium were poured into culture bottles, the media were sterilized by autoclaving for 15 minutes at 121^oC and a pressure of 1.05kg/cm^2 (Peddabonia, 2006), and transferred into biosafety cabinet to cool for culturing.

Plate 2: Prepared media for study

Local seeds of pepper varieties were washed under running tap to remove dirts, using morning fresh, seeds were soaked in warm water for 10 minutes due to its recalcitrance, under the laminar flow hood, seeds were transferred into a sterile flask and soaked in 70% ethanol for 10 seconds, the ethanol was decanted and the seeds were rinsed thoroughly, and were immersed in 20% sodium hypochloride (NaOCl) for 10 minutes, 2 drops of tween 20 were added and rinsed three (3) times with sterile distilled water, the seeds were picked wit autoclaved flamed forceps (sterile) and placed on the cutting board to dry.

Sterilized seeds were cultured in prepared medium (Murashige *et al.,* 1962). Cultures were kept for 3 days under continuous dark, then transferred to 16 hours photoperiod per day at light intensity of 17.7µ mol/m^2/s provided by cool white fluorescent tubes and temperature were set at 25^{o}C.

Cotyledons excised from germinated seedlings were cut into small parts each measuring about 0.25cm^2. Explants were cultured into ordinary MS media (control) and MS supplemented with each of the growth hormones (treatment) that is; IBA, GA_3, $AgNO_3$ and Nicotinic acid at a specific concentration. Explants were subcultured at two weeks intervals into the same medium (Dabauza and Pena, 2001). Explants with roots were acclimatized for 48 hours after washing off the Agar (gelrite) with deionized water to pots with a mixture of substrate and perlite (5:1) in a screen house and transferred into green house, were they developed into normal plants and bearing normal fruits.

Plate 3: Cultures kept in the dark

Plate 4: Geminated seeds after 2 weeks of culture

Data were collected for germination after two weeks after planting in pots, Shoot elongation after six weeks after planting in pots, Rooting capacity (number of roots) after four week after planting in pots and fruit yield after eight weeks after planting in pots. Mean and mean effect of the result were calculated and subjected to analysis of variance using (ANOVA), treatment means were separated using the least significant difference (LSD) at $p<0.05$.

DISCUSSION

The results of the study revealed that treatments significantly affected germination of pepper varieties ($p<0.05$) (Table 1). IBA showed the highest number of germination at concentration of 2.5ml in all pepper varieties, GA_3 showed highest number of germination in concentration of 5ml in Atarugu, 3.8ml concentration in Tatashi, Borkonu and Shumbo probably because of the variation in pepper type. In Nicotinic acid, germination rate was highest in concentration of 1.5ml on Borkonu, Shumbo and Tatashi but showed highest yield of germination on Atarugu at 2.5ml concentration and in $AgNO_3$, the highest yield of germination rate of the four pepper varieties was obtained in 1ml concentration of $AgNO_3$.

On the basis of rooting capacity, the four treatments significantly ($p<0.05$) promoted the roots of the pepper varieties at different concentration though some concentrations enhanced rooting more than others (Table 2). The highest yield of roots were obtain in concentration 2.5ml IBA, 6.3ml GA_3 on Atarugu, Borkonu, Shumbo and 5ml GA_3 on Tatashi, 2.5ml Nicotinic acid on Atarugu and Shumbo, 3ml Nitotinic acid on Tatashi and Borkonu. $AgNO_3$ on the other hand significantly promoted rooting ($p<0.05$) capacity in all pepper types but the highest root was obtained on 2ml $AgNO_3$ concentration.

The shoot elongation of pepper varieties was obtained in Basal medium supplemented with different concentration of IBA (Table 3) but the highest yield of elongated shoots in IBA was obtained in 2.5ml concentration in the four pepper varieties which is in accordance with those reported in some studies (Dabauza and Pena, 2001; Peddaboina *et al.*, 2006; Guadalupe *et al.*, 2009). GA_3 also induced shoot elongation in all pepper varieties and the best result was obtained with the medium supplemented with 5ml GA_3 in all pepper varieties.

$AgNO_3$ also promoted shoot elongation of the four pepper varieties at different concentrations but the highest elongated shoot were obtained at concentration of 1ml $AgNO_3$ in all the pepper types which is in agreement with (Chen Qin *et al.*, 2005; Mezghani *et al.*, 2007; Ashrafuzzaman *et al.*, 2009) that found Ms with GA_3 or $AgNO_3$ to be the best elongation medium. Nicotinic and also showed significant variation in the promotion of shoot elongation of the pepper varieties at different concentration, that is in Atarugu, the highest yield of elongated shoot was obtained from concentration 3ml Nicotonic acidic, while inn Borkonu, Shumbo and Tatashi, the highest shoot elongation was obtained from concentration of 1.5ml Nicotinic acid (Chen Qin *et al.*, 2005).

Plate 5: explants from different concentrations

Plate 6: Roots of explants

Table 1: Efficacy of PGRs (mg/ml) on germination(%) of different pepper varieties.

Trt	MS	MS+IBA				MS+GA_3				MS+$AgNo_3$				MS+Nicotinic acid			
	0.0	0.3	1.3	2.5	3.8	2.5	3.8	5.0	6.3	0.5	1.0	1.2	2.0	1.5	2.0	2.5	3.0
Ata	4.7	8.7	8.3	14.0	9.7	7.0	12.7	13.3	9.3	8.7	16.7	12.0	10.0	15.3	8.7	9.0	9.0
Bor	6.0	8.3	8.3	13.7	8.0	8.7	10.7	10.7	8.3	10.3	16.0	11.7	10.3	13.3	9.3	10.0	9.7
Sh	5.3	6.7	7.0	11.0	9.3	7.7	9.7	10.7	10.7	9.7	15.3	11.3	9.7	12.7	8.3	9.7	9.3
Ta	4.3	6.0	5.3	10.3	8.7	8.7	9.3	9.3	8.3	9.0	14.7	12.3	10.0	11.7	8.7	8.7	8.0

$LSD_{0.05}$ =1.50

Trt= Treatment, Ata = Atarugu, Bor = Borkonu, Sh = Shombo, Ta = Tatashe. Any difference between a pair of treatment mean that is higher than 1.50 is considered to be significant at 5% level.

The result showed that there is significant different among treatment among treatment means

Table 2: Efficacy of PGRs (mg/ml) on rooting capacity (number) of different pepper varieties (%)

Trt	MS	MS+IBA				MS+GA_3				MS+$AgNo_3$				MS+Nicotinic acid			
	0.0	0.3	1.3	2.5	3.8	2.5	3.8	5.0	6.3	0.5	1.0	1.2	2.0	1.5	2.0	2.5	3.0
Ata	3.0	6.3	6.0	7.3	5.7	6.0	6.7	7.0	9.7	5.0	6.3	7.3	7.7	5.3	6.7	7.7	6.0
Bor	3.0	5.7	5.7	6.7	5.0	5.0	5.0	9.0	9.3	4.0	4.7	4.3	5.3	4.7	5.0	6.0	6.0
Sh	3.0	4.0	4.7	5.0	4.7	4.7	6.3	8.0	8.7	4.7	5.0	5.3	6.0	4.3	5.7	7.3	6.7
Ta	4.0	4.3	5.3	6.3	4.3	7.0	8.0	8.0	6.3	4.0	6.0	5.3	7.0	3.7	5.7	7.0	7.3

$LSD_{0.05}$ = 0.46

Trt= Treatment, Ata = Atarugu, Bor = Borkonu, Sh = Shombo, Ta = Tatashe. Any difference between a pair of treatment means, that is higher than 0.46 is considered to be significant at 5% level.

The result showed that there is significant different among treatment among treatment means.

Table 3: Efficacy of PGRs (mg/ml) on shoot elongation of different pepper varieties (cm)

Trt	MS	MS+IBA				MS+GA_3				MS+$AgNo_3$				MS+Nicotinic acid			
	0.0	0.3	1.3	2.5	3.8	2.5	3.8	5.0	6.3	0.5	1.0	1.2	2.0	1.5	2.0	2.5	3.0
Ata	17.7	26.6	27.9	28.3	24.5	32.0	32.8	57.7	39.7	42.9	54.0	39.3	31.9	25.4	27.2	28.9	31.1
Bor	17.7	25.4	26.3	29.5	26.5	35.8	46.7	62.6	52.6	42.0	56.0	39.7	33.5	34.2	30.5	30.5	26.1
Sh	17.7	26.7	21.8	31.8	29.1	46.4	49.5	57.3	50.3	36.3	52.7	39.7	33.5	32.6	24.7	25.9	23.6
Ta	17.7	20.7	23.3	20.0	30.7	32.7	39.8	32.9	39.2	51.7	41.3	26.9	24.2	20.1	22.3	22.3	21.6

$LSD_{0.05}$ = 0.46

Trt= Treatment, Ata = Atarugu, Bor = Borkonu, Sh = Shombo, Ta = Tatashe. Any difference between a pair of treatment mean, that is higher than 0.46 is considered to be significant at 5% level.

The result showed that there is significant different among treatment among treatment means.

Table 4: Efficacy of PGRs (mg/ml) on number of fruit yield per pepper variety (%)

Trt	MS	MS + IBA				MS + GA_3				MS + $AgNo_3$				MS+Nicotinic acid			
	0.0	0.3	1.3	2.5	3.8	2.5	3.8	5.0	6.3	0.5	1.0	1.2	2.0	1.5	2.0	2.5	3.0
Ata	2.7	4.3	9.3	11.3	7.7	5.0	6.7	8.7	4.0	11.0	9.0	6.7	5.0	6.0	8.0	15.7	11.3
Bor	2.7	5.3	13.0	15.7	15.7	10.0	11.0	16.0	8.7	18.7	5.0	5.0	6.0	8.0	9.7	22.0	14.7
Sh	2.7	6.0	8.3	11.3	9.7	7.0	7.7	9.3	6.3	9.3	7.0	6.0	7.0	5.3	5.7	9.3	7.0
Ta	2.7	5.0	6.1	7.7	7.0	4.0	4.7	8.0	5.3	4.0	4.0	6.3	8.0	5.0	6.7	8.3	8.0

$LSD_{0.05}$ =0.61

Trt= Treatment, Ata = Atarugu, Bor = Borkonu, Sh = Shombo, Ta = Tatashe

Any difference between a pair of treatment mean that is higher than 0.61is considered to be highly significant at 5% level.

The result showed that there is significant different among treatment among treatment means.

Plate 7: Elongated roots of explants in screen house

Plate 8: Some explants with fruits (arrow)

Supplementing MS basal medium with different PGRs significantly enhanced fruiting of pepper varieties (Table 4). IBA gave the highest yield at concentration 2.5ml in all pepper types, in treatment with GA_3, the highest number of fruit was observed at 5ml GA_3 concentration, in treatment with Nicotinic acid, the highest number of fruit was observed at concentration 2.5ml in Atarugu, Shumbo and Tatashi, and at 3ml concentration on Borkonu. $AgNO_3$ also had the highest number of fruit observed at concentration 0.5ml on Atarugu, Borkonu and Shumbo and showed a difference at higher yield in Tatashi at 8ml $AgNO_3$ concentration which could be due to pepper type.

CONCLUSION

In conclusion, four the local pepper varieties were found to regenerate on proper medium, but they have difference in differentiation rates and yields resulting from genotype, explants type, seedling stage and ingredients in the media (Chen Qin et al.,, 2005). The best formula for all the medium used for germination test in this study is MS + 1ml $AgNO_3$ which made explants germinate and differentiate at high rates with good development.

Hyde *et al.,* (1996) observed that $AgNO_3$ influenced shoot induction of two cultivars. Explants were not able to elongate at high rate in the medium containing only MS without plant regulators, $AgNO_3$ was optimum in all pepper varieties, in order to confirm whether the action of $AgNO_3$ is affected by gene type or not, $AgNO_3$ was regarded as an induction promotion factor added to the medium. According to (Vain *et al.,* 1989), $AgNO_3$ was an ethylene inhibitor. Ethylene suppresses callus to differentiate and $AgNO_3$ climates the ethylene action, which in return favours explants differentiation.

In the present study, GA_3 promoted shoot elongation that is to say GA_3 is an elongation – promotion factor.

Though work has been carried out on pepper treated with different growth hormone, no work has been done on local peppers in Keffi, we therefore recommend that more work should be carried out to improve on the *in vitro* yield and propagation of local pepper varieties in Keffi.

REFERENCES

Ashrafruzzaman M, Hossain M.M, Razi ismail M , shahidul Haque M, Shahidullah S.M, Shahin- Uz-Zaman (2009). Regeneration potential of seedling explants of chili (*Capsicum annuum* L.). *African Journal of Biotechnology* Vol: 8(4) Pp.591-596.

Bosland, P.W. (1994), chiles history, cultivation and uses in species, herbs and edible fungi, *charalambous, G (Ed)* Elsevier pub. Pp. 347-366.

Chen Qin, Dong Zhao-long, Liu Wen-Xuan , Deng Zhi-rui, Tang Liang (2005). Effect of exogenous plant growth regulators on in- vitro regeneration of cotyledon explants in pepper. Notulae botaniccae Horti Agrobotanici Cluj-Napocca 33(1). Pp 25-32.

Dabauza, M. and Pena, L., (2001), High efficiency organogenesis in sweet pepper (*capsicum annuum* L.) tissue from different seedling explants, *plant growth regulator*, 33, 221-229.

Diaz, I., Moreno, R. and Power, J.B. (2000), Plant regeneration from protoplasts of *Capcicum annuum Plant Cell Reproduction*, 7:210-212.

Hyde, C.C. and Philips, G.C. (1996), Silver nitrate promotes shoot development and plant regeneration of chile pepper (*Capsicum annuum* L.) via organogenesis, *In vitro Cell Dev. Biol. Plant*, 32, 72-80.

Mezghani (2007) Adventitious shoot bud induction in chili pepper. Pp.213-215.

Morrison, R.A., Koning, R.E. and Evans, D.A. (1962), Pepper In: Evans, D. A., Sharp, W.R., Ammirato, P.V. eds. Handbook of plant cell cultures, vol. 4 New York: Macmillan. 552-573.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures, *physio, Plant, 5,* 473-497.

Peddabonia, V., Thamidal, C. and Karampuri, S. (2006), *In vitro* shoot multiplication and plant regeneration in four *Capsicum* species using thidiazuron. Scientia Horticulture 107:117 - 122.

Vain, P., Yeen, H., Flament. P. (1989), Enhancement of production and regeneration of embryogenic type callus in *Zea mays* L. by $AgNO_3$, *Plant Cell Tissue Organ Culture,*18, 143-151.